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# Zooplankton as a Food Source

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# ZOOPLANKTON AS A FOOD SOURCE

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16. ABSTRACT

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An Artemia reference sample is now available, so that one can compare the growth and survival of experimental animals fed the reference brine shrimp versus those fed an acquired batch of Artemia.

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ABSTRACT

While thousands of zooplankton species could potentially serve as food for larval stages of cultured fish and crustaceans, the aquaculturists practical choice of a reproducible live food ration is quite limited. In practice, rotifers and brine shrimp are the most commonly used zooplankton for these critical larval stages. The reasons for the popularity of these organisms lies in the fact that they are easily obtained or cultured, are of appropriate size, and have been shown to support a wide spectrum of larval forms. Recently, aquaculturists have turned their attention to the quality of the rotifers and brine shrimp that are being fed. A number of papers have reported the success of rearing a larval fish or shellfish on one batch of rotifer or brine shrimp, but not on another. It has been well documented that the nutrition and environment of the zooplankton has a profound effect on the predatory organism. The brine shrimp has been shown to have a limited capacity to biosynthesize long chain fatty acids and, thus, its nutrition takes on added significance. Also, Artemia have been shown to readily bioaccumulate chlorinated hydrocarbons from the water and pass these on to the predator.

An Artemia reference sample is now available, so that one can compare the growth and survival of experimental animals fed the reference brine shrimp versus those fed an acquired batch of Artemia.

## INTRODUCTION

The word, plankton, is derived from a Greek work meaning, to wander. Zooplankton, referring specifically to microscopic animal life floating or drifing in water, serves as the primary source of food for larval stages of many aquatic species of economic importance. The usual list of zooplankton organisms includes protozoans, rotifers, brachiopods, copepods and invertebrate larvae. Thus, thousands of animals could serve as potential food for cultured fish and shellfish.

Since the culture of laboratory animals requires a stable, reproducible ration, the number of zooplankton species of potential use to culturists rapidly shrinks to a few standard organisms. In actual practice, aquaculturists depend heavily on rotifers and brine shrimp to support critical phases of larval development, though neither of these organisms is found in significant numbers in the sea. The reason for the popularity of these organisms lies in the fact that both are easily cultured or obtained, and have been shown to support a very wide variety of larval forms. Comprehensive bibliographies are available on both Artemia (Sorgeloos et al. 1980a) and Brachionus (Solangi and Ogle 1977). The treatise edited by Kinne (1977) should be consulted for the cultivation of zooplankton in general.

The present paper will focus on the nutritional aspects of zooplankton (proteins, lipids, etc.), and some non-nutritional factors (size, contamination, etc.). The ultimate goal of an aquacultural feed is to provide for rapid growth and high survival of the cultured organism. It is also important, for consumer

acceptance, that the aquaculturally produced animals resemble the wild organism in such characteristics as color, taste, texture, and size.

What has been "discovered" recently is that Artemia, rotifers, etc. are living organisms and can vary with environmental conditions. Thus, in describing the diet of an animal, to say, "Artemia was fed", is not equivalent to saying, "casein was fed." Examples will be given in this paper to show that some sources of rotifers, Artemia, etc. support good survival and growth whereas others cause 100% mortality of larvae. The effect is obvious -- the cause is slowly being understood.

## SIZE

The suitability of zooplankton for early life stages may be directly related to size. The live food may be of good nutritional value but not practical because it is too large to be ingested by larvae. Sulkin and Epifanio (1975) compared rotifers, Artemia nauplii, and sea urchin gastrulae as food for the first and second zoeal stages of the blue crab (Callinectes sapidus Rathbun). The rotifers used in this experiment ranged in size between 45 and 180 µm, whereas the "recently hatched" Artemia nauplii were 250 µm in width. These authors state that 110 µm is the maximum size for the food of the early blue crab larvae.

Brachionus rotifer strains vary in size. A Japanese strain ranges in size from 74-124 µm (Ito 1960), and a California strain from 66-182 µm (Theilacker and McMaster 1971). The biometrics of Artemia strains from different geographic origins have been

reported by Vanhaecke and Sorgeloos (1980). It was found that there was a large variation between strains and little difference within strains. Beck and Bengtson (in press) reported the effect of size in eight geographical strains of brine shrimp ranging from 433 ± 21 µm to 515 ± 37 µm in length. A direct relationship was found between increased brine shrimp size and mortality of Atlantic sliverside (Menidia menidia) larvae fed the various strains (Figure 1).

Data regarding size and suitability of a live organism as a first food are primarily from studies with fish. For example, several types of live organisms have been cultured as first food for small larval fish. Trochophore larvae of oysters (Crassostrea virginica) were used to rear turbot, Scophthalmus maximus (Howell 1973). Veliger larvae of opistobranch molluscs (90-140 µm) have been used successfully to feed northern anchovy, Engraulis mordax, in combination with a naked dinoflagellate, Gymnodinium splendens (Lasker et al. 1970). The anchovy larvae selected prey in the size range 50-90 µm when first feeding.

Copepods were fed to first feeding scaled sardines, Harengula pensacolae, and bay anchovy, Anchoa mitchilli. Scaled sardines, which began feeding at 4.5 mm total length, selected copepodites averaging 63 µm width while bay anchovy, which began feeding at 3.4 mm total length, selected copepodites averaging 58 µm in width (Detwyler and Houde 1970). Houde (1973) stated that, according to his experience, most fish larvae, including those with relatively large mouths, began feeding on organisms in the 50-100 µm width range. Copepod nauplii and molluscan larvae fall within this

range as does the rotifer, Brachionus plicatilis, one of the most important food organisms used in culturing small-mouthed larval fish.

Most decapod crustacean larvae can be reared on Artemia nauplii for their complete development (Rice and Williamson 1970; Provenzano and Goy 1976). Some examples are American lobster, Homarus americanus (Carlberg and Van Olst 1975), stone crab, Menippe mercenaria (Mootz and Epifano 1974), grass shrimp, Palaemonetes pugio (Provenzano and Goy 1976), and Cancer irroratus (Sastry 1970). The exceptions appear to be blue crab, Callinectes sapidus, mentioned previously (Sulkin and Epifanio 1975), and penaeid shrimp which are fed various species of algae (Hudinga and Miyamura 1962; Griffith et al. 1973). Mock et al. (1980) used active dry baker's yeast as a replacement for algae with some success for feeding Penaeus stylirostris. When the shrimp metamorphosed to mysis I stage, they were fed frozen Artemia nauplii and then switched to live Artemia nauplii at the end of mysis II stage.

## PROTEIN

It is generally assumed that a live food should provide protein levels and amino acid profiles similar to that found in the cultured or wild organism. A second approach is to pattern the amino acid profiles after the food considered best for that organism. In a series of elegant experiments based on this concept, Deshimaru and Yone (1978) reported on an "artificial clam" diet for the prawn, Penaeus japonicus. The optimum dietary

level of protein was in the range of 52-57%. Other penaeid shrimp have been reported to require a diet containing less than 40% total protein, e.g. Penaeus setiferus (Andrews et al. 1972), P. aztecus (Venkataramiah et al. 1975), and P. indicus (Colvin 1976).

Potential shrimp feed shown to contain approximately 50% protein include rotifers cultured on algae (Scott and Baynes 1978), brine shrimp nauplii (Schauer et al. 1979), and brine shrimp adults (Deshimaru and Shigeno 1972; Gallagher and Brown 1975).

Although more is known about the amino acid requirements for fresh water and marine fish than shellfish (cf. Cowey 1979), some basic work has been accomplished in prawn nutrition. Deshimaru and Shigeno (1972) found that diets similar to P. japonicus in amino acid profiles, such as Artemia, squid, and short neck clams supported good growth. As part of the same study, white fish meal-based diets were found to give poor growth. These authors concluded that a protein source lacking sufficient levels of basic amino acids (e.g. lysinne, arginine and histidine) supported inefficient growth. They listed fish meal as a poor source of the basic amino acids.

Table I lists the amino acid profiles of several zooplankton species. Brachionus, Acartia, Tigriopus, Moina and Penaeus show very similar amino acid profiles. Artemia nauplii amino acid patterns show the greatest variation. Seidel et al. (1980a) compared the amino acid profiles and also found some variation between the nauplii from Shark Bay, Australia, Macau, Brazil, San Pablo Bay, California, the Great Salt Lake, Utah and Margherita di

Savoia, Italy. These differnces in profiles among strains were less, however, than those reported in Table I, possibly showing variation in laboratory techniques. Seidel et al. (1980a) concluded that all five geographical strains could meet the essential amino acid requirements as established for Chinook salmon. Gallagher and Brown (1975) concluded that "the brine shrimp values compare favorably with those for both casein and egg albumin." On the basis of PER and NPU values and protein digestibility, Watanabe et al. (1978a), concluded that Brachionus, Artemia, Tigriopus, Moina and Daphnia are superior food protein sources for fish.

Diet appears to have little effect on the amino acid composition of the live food (Watanabe et al. 1978a; Claus et al. 1979), and in turn, amino acid composition of the predator (Seidel et al. 1980b).

## LIPIDS

The lipid content of a food is an important factor in the value of the food. In practice, the term lipid denotes any material extracted by an organic solvent such as chloroform. Thus, crude lipid would include glycerides, steroids, waxes, carotenoids, phospholipids, etc. Triglycerides, waxes and phospholipds constitute the major source of metabolizable energy as well as a source of essential fatty acids.

Waxes are esters of long chain alchols and fatty acids. They have been shown to be a common storage lipid for copepods in temperate and polar environments where lipid storage is more pronounced (Lee and Hirota 1973). As a reserve energy source, the

waxes may represent a level of greater than 50% of the total lipid present (Lee 1975). Copepods from tropical and subtropical areas were characterized by having fewer storage lipids, including waxes. It appears that the waxes represent a de novo synthesis by the arctic copepods (Sargent and Lee 1975).

While wax-containing copepods are abundant, little is known about the assimilation of the waxes by predators. It has been reported that trout and herring metabolize dietary wax esters and deposit triacylglycerols in their bodies (Sargent et al. 1979). Sargent et al. (1979) and Patton and Benson (1975) conclude that "wax esters are very efficienttly assimilated in the marine food-chain."

As with proteins, more is known on the essential fatty acid requirements of fish than shellfish. Nevertheless, a very similar picture is emerging in regard to the need for these fatty acids in the diet. Unlike protein/amino acid patterns, the fatty acid profiles found in the cultured organism often closely resemble the phytoplankton it feeds upon. Thus, the fatty acid profile of the rotifer, Artemia, or copepod may be significantly changed by diet and could be of good or poor nutritional value.

Generally, Artemia cysts are purchased whereas rotifers are cultured in the laboratory. The Artemia cysts would presumably resemble the adult Artemia in fatty acid composition while the rotifer would resemble the culture diet. The need for essential fatty acids in the diet of rotifers has become apparent and a similar picture for Artemia has emerged.

Scott and Middleton (1979) cultured Brachionus plicatilis on

on several different unicellular algae at three different temperatures. The rotifers generally had fatty acid profiles like their algal food. The Dunaliella-fed rotifers were high in 18:3ω3 and low in 20:5ω3 and 22:6ω3. The Isochrysis, and especially the Paulova and Phaedactylum-fed rotifers contained less 18:3ω3 and more 20:5ω3 and 22:6ω3. The turbot larvae fed the Dunaliella-fed rotifers exhibited stunted growth and high mortalities. Turbot larvae fed Dunaliella-fed rotifers plus other algae-fed rotifers showed good survival and growth, revealing an apparent nutritional problem rather than a toxic effect.

A very similar picture has emerged from the work on rotifers in Japan (c.f. Fujita 1979). Because of the great demand for rotifers, baker's yearst was substituted for Chlorella as a rotifer feed and the result was high mortalities in sea bream (Watanabe et al. 1979; Kitajima et al. 1980a), and ayu, Plecoglossus altivelis (Kitajima et al. 1980b). A number of experiments were reported with a so-called ω-yeast. The ω-yeast was prepared by culturing baker's yeast in a medium supplemented with fish oils. The resulting rotifers were found to have a higher level of ω3 HUFA's (highly unsaturated fatty acid), and to support good survival and growth of red sea bream (Kitajima et al. 1980a), and P. altivelis (Kitajima et al. 1980b).

Watanabe et al. (1978b) analyzed the fatty acid content of brine shrimp from San Francisco, South America and Canada. These authors classified Artemia on the basis of their fatty acid profiles. They grouped those with high 18:3ω3 levels as being suitable for fresh water fish only, and those with high 20:5ω3 as

being most suitable for marine fish. In a later report, Watanabe et al. (1980) classified Artemia cysts from San Francisco, China, Canada and West Germany into the same two categories. The San Francisco strain was shown to fall into both classes, depending on the year of harvest. An Artemia with a high 18:3ω3 and low 20:5ω3 content was consistently found, by these authors, not to support growth and survival of sea bream larvae.

Schauer et al. (1980) reported on the fatty acid profiles of three San Francisco Bay area Artemia collections, and on strains from Australia, Brazil, Italy and Utah (Table II). As can be seen, San Pablo Bay (SPB 1628) and Utah (1977) are high in 18:3ω3 and low in 20:5ω3. One of the San Francisco collections (SF 313) was found to be intermediate between the San Pablo Bay (SPB 1628) and Utah, and the other strains. The most significant variation was in the survival and growth of various fish and shellfish larvae on the various strains. This will be discussed in detail below.

Moreno et al. (1979a) suggest that phytoplankton are able to perform de novo synthesis of all their fatty acids. These authors showed that the diatom, Phaeodactylum tricornutum, transformed radioactive saturated and unsaturated precursors into 20:5ω3 and 22:6ω3 fatty acids. Phytoplankton that have high concentrations of 20:5ω3 and 22:6ω3 fatty acids would contribute them to the marine food web. Many marine species have been shown to require these polyunsaturated fatty acids in their diet. This requirement is largely based upon the ability of the organism to biosynthesize the 20 and 22-carbon fatty acids from 18-carbon analogs.

The calanoid copepod, Paracalanus parva was shown by Moreno et al. (1979b) to be able to form ω9 fatty acids and synthesize long chain fatty acids. P. parva was shown to be incapable of a synthesis of linoleic or linolenic acids. Schauer (personal communication) incorporated 1-$^{14}$C 18:3ω3 into a rice bran diet for Artemia. The Artemia were then fed to rainbow trout and winter flounder (Pseudopleuronectes americanus). As noted in Table III, 1-$^{14}$C 18:3ω3 was the only ω3 fatty acid in the diet. Artemia were shown to form 20:5ω3 and 22:6ω3 fatty acids from 18:3ω3. While 22:6ω3 is not commonly isolated from nauplii, we have found it in adult Artemia. These data show that Artemia can make HUFA's from 18:3ω3, probably in sufficient amounts to satisfy their needs. The results shown in Table II relfect a combination of fatty acid profiles resulting from biosynthesis and the particular diet available at the time to the Artemia in that geographical region. The ability of some marine crustaceans to biosynthesize 20:5ω3 and 22:6ω3 from palmitate was also reported by Morris and Sargent (1973). Table III further shows that rainbow trout were able to form 20:5ω3 and 22:6ω3, whereas winter flounder contained approximately the same amounts of these fatty acids at 24 hours as were in the Artemia.

Kanazawa et al. (1979) injected 11-$^{14}$C 18:3ω3 fatty acids into several different marine species in order to compare their ability to form 20:5ω3 and 22:6ω3 fatty acids. Table IV shows the conversion relative to rainbow trout. A number of authors have shown the ability of the freshwater fish to convert 18:3ω3 to HUFA's (cf. Kanazawa et al. 1979, rainbow trout). Kanazawa et al.

(1979) concluded that the marine animals in their study were incapable of synthesizing enough 20:5ω3 and 22:6ω3 from 18:3ω3 to satisfy their dietary requirements. Penaeus japonicus was listed as moderate in this biosynthetic capacity.

Table V shows the fatty acid profiles of San Pablo and Brazil Artemia nauplii which were cultured in ponds at SEAFDEC (South East Asia Fisheries Development Center), Philippines. The harvested adult profiles were similar to each other, very different from the parent cysts, and probably resembled the phytoplankton in the pond.

Sorgeloos et al. (1980b) fed defatted rice bran to Artemia and reported a good protein recovery in the Artemia. However, the fatty acid profile showed very low levels of HUFA's. Thus, if the adult Artemia is to be fed to marine species, biomass is not as important a consideration as fatty acid profiles, and the warnings of Watanabe et al. (1980) need to be heeded.

## CAROTENOIDS

Zooplankton such as the copepods and Artemia are pigmented with carotenoids. These lipids may be deposited in the predator or, in some cases, assimilated as Vitamin A precursors. Astaxanthin is the major crustacean pigment, although canthaxanthin is the major carotenoid present in a variety of geographical strains of Artemia (Soejima et al. 1980). When incorporated into their diet, a number of carotenoids, ordinarily not isolated from Artemia, were found (Soejima et al. 1980). Table VI shows that effect of feeding Artemia a diet of Spirulina, containing 25% β-

carotene and 65% zeaxanthin.

Colorless lobsters, crabs, prawn, and other crustaceans can be produced by feeding a carotenoid-free diet. Nevertheless, consumer acceptance is based partly on color of the marketed food product. For example, when purchasing Penaeus japonicus live, Japanese consumers demand a red, not a blue-colored prawn. The feeding of pigmented zooplankton has been shown to be effective in providing proper pigmentation (cf. Sargent et al. 1979; Simpson et al. 1981).

## MINERALS

Several authors have reported the mineral composition of zooplankton (Gallagher and Brown 1975; Watanabe et al. 1978c; Olney et al. 1980). Watanabe et al. (1978c) fed Tigriopus, Moina and rotifers several live diets and found no significant differences in the mineral content of the cultured organisms. Table VII shows the mineral composition of Artemia cysts and nauplii taken from five geographical locations. The twelve metals were measured by atomic absorption and neutron activation analysis. While large variations were seen in the metal content of the cysts, the nauplii showed lower levels and less variation between strains.

Watanabe et al. (1978c) compared the mineral content of a single strain of Artemia hatched in three different locations, and reported similar levels of the minerals tested. Watanabe et al. (1978c) reported a slight seasonal variation in the mineral content of Arcatia clausi. Watanabe et al. (1978c) concluded that

"minerals are not the principle factors in the dietary value of living feeds." Olney et al. (1980) further concluded that the differences in survival and growth obtained by feeding certain geographical strains to several fishes and invertebrates was not related to the mineral content analyzed.

## VITAMINS

Data on the vitamin content of zooplankton appears to be limited to essentially a single report on brine shrimp by Gallagher and Brown (1975). These data are presented in Table VIII.

## CONTAMINANTS

Since the publication of Bookhout and Costlow (1970), attention has been drawn to the fact that live food can carry adventitious materials to the predator. Most commonly noted, perhaps, is the role of the persistent chlorinated hydrocarbons. Less attention has been given to heavy metals, biotoxins or microorganisms that might be carried by the zooplankton.

The paper by Bookhout and Costlow (1970) compared the ability of Artemia nauplii from Utah and San Francisco to support growth and survival of four crab species. It was consistently found that the Utah Artemia were not as good as those from San Francisco. It was reported that the Utah Artemia had three times the level of DDT (dichlorodiphenyltrichoroethane) as that contained in the San Francisco strain; the higher level of DDT was suggested as the cause of larval crab mortalities. A short time after these data

appeared, Wickens (1972) reported lower levels of DDT in the Utah Artemia.

In 1980, Olney et al. analyzed the chlorinated hydrocarbon content of five geographical strains of Artemia. it can be seen from Table IX that there is much variation between sample sites (Australia, Brazil, Italy, Utah, California) and some variation between San Francisco Bay and San Pablo Bay. The five strains of Artemia were tested on winter flounder (Klein-MacPhee et al. 1980) and Atlantic silversides (Menidia menidia) (Beck et al. 1980). In both cases, Utah and San Pablo Bay Artemia gave poor results. Olney et al. (1980) reported that these fish accumulated some chlordane, dieldrin, DDT and PCB's, (polychlorinated biphenyls) and suggested that these could be the cause of poor results for San Pablo Artemia. However, in Table IX the contamination and accumulation data for Utah are not high and would seem to offer no explanation for the poor survival with the Utah diet.

In an attempt to delineate the possible causative role of chlordane and dieldrin, McLean (1980) contaminated Artemia with these two compounds at physiological levels. In a preliminary experiment, defatted rice bran was contaminated with the pesticides and fed to Artemia. The Artemia produced by this procedure showed a low bioaccumulation factor. Rearing the nauplii in water contaminated with the pesticides resulted in higher tissue residues in a shorter time, thus providing suitably-sized nauplii for feeding. The treatments included controls and various combinations of low contamination (0.1 ppb) and high contamination (1.0 ppb) of chlordane and dieldrin. Table

X shows that Artemia readily bioconcentrate these two pesticides. When Artemia from Brazil, with various levels of contamination, were fed to winter flounder, significant changes in fish length were noted that were directly related to the chlorinated hydrocarbon content. Although no mortalities were noted, it should be stressed that due to the size of the contaminated Artemia nauplii, the winter flounder were post-metamorphosis.

In a similar study (Johns et al. in press), contaminated Artemia were fed to Rhithropanopeus harrisii larvae. No adverse effect on growth or survival in the mud crabs was noted. These results suggest that the contamination levels reported by Olney et al. (1980) and Bookhout and Costlow (1970) do not totally explain the adverse effect noted with certain Artemia predators.

Schauer et al. (1980) suggested that a poor fatty acid profile and a "high" level of contamination may give the poor survival and growth noted in these studies. The controlled feeding of a poor fatty acid profile and contaminated Artemia to either crab or winter flounder larvae has not been reported.

It should be noted that an analysis of the chlorinated hydrocarbons in Utah Artemia (1965-1978) has shown a steady improvement (Seidel, personal communication) (Table XI), although the 1977 and 1978 lots of Artemia from utah gave poor results with R. harrisii larvae (Johns et al. 1980).

The results of the various feeding trials of the five geogrpahical strains showed some variation as to the ranking of the Artemia cysts. The results are tabulated in Table XII. It should be stressed that these results apply to only the particular

batches tested, as we have noted significant variations between batches from the same source.

## ENZYMES

It is a common experience among aquaculturists that there are critical stages of larval fish growth which, to date, are supplied only by live food. Artificial diets have been constructed which are similar in nutrients and size (microcapsules) yet seem to fall short of giving the survival and growth of the live diet (cf. Seidel et al. 1980b). An obvious problem with the artificial diet is that of the leaching of nutrients and subsequent uncontrolled bacterial growth. This problem will be enventually addressed through improved microencapsulation techniques. A second possible advantage of the live diet is that the live food contains exogenous digestive enzymes which may supplement and/or activate endogenous enzymes of the larval species (Dabrowski and Glogowski, 1977).

Samain et al. (1980) determined the levels of trypsin and amylase in Artemia during the first 24 days after hatching. The Artemia were fed on various phytoplankton in these studies. While the amylase and trypsin levels were seen to rise between day 1 and day 2, these levels were low in comparison to the later specific activities of these enzymes. Moreover, the activities were reported to be controlled by the food levels and the food composition. Thus, the non-feeding instar I through III Artemia nauplius was shown to be low in these two enzymes. Benijts et al. (1975) showed that the instar I stage of Artemia as superior to

later stages as a feed from a caloric point of view and Clau et al. (1979) reported the same results from their growth experiements.

Seidel et al. (1980b) compared the growth and survival of Atlantic silverside larvae on a live 3-day-old brine shrimp diet, an artificial diet, and a combination of the two diets. While the artificial diet alone gave poor results, a combination of Artemia on one day and the artificial diet for the next 7 days gave nearly comparable results to a 100% Artemia diet. These results would tend to rule out the digestive enzymes of brine shrimp as its main advantage as food for Atlantic silverside larvae. In the same study, Seidel et al. (1980b) observed that freeze-dried Artemia did not support fish growth and survival as well as live Artemia. Grabner et al. (1981) studied the suitability of freeze-dried and frozen zooplankton for larval fish. The two processes (freezing and freeze-drying) had little effect on the enzymes tested but did damage the cells, causing significant leaching of enzymes into the medium. In addition, free amino acids were found to be readily lost and one would expect a simultaneous loss of water soluble vitamins, had they been measured.

Studies on the role of exogenous digestive enzymes supplied from a live food source, in the digestion processes of predators are underway in our laboratory at this time.

## CONCLUSIONS

The value of zooplankton in the nutrition of larval forms of shellfish and finfish has been well established. In the last

decade, we have become aware of the fact tthat there are significant physical, chemical and biological variations between, and among strains of zooplankton. These variations are of genetic and environmental origin. While the protein level is generally similar, large variations have been shown in the size, fatty acid profiles and contamination of the cultured zooplankton. Nevertheless, the literature still contains references that state only that "a given zooplankton was fed," as though these are defined diets.

The analysis of the available Artemia strains for selected chemical, biochemical, morphological, and genetic characteristics, and the cultivation response of a predator is clearly beyond the capability of a single laboratory. In 1977, the International Study of Artemia (ISA) group was formed with the purpose of characterizing five geographical strains of Artemia. Much of the research cited in this paper has been drawn from approximately 30 ISA papers. The complete analysis of a single lot of Artemia has allowed comparisons of data to be made which are not possible with much of the literature on zooplankton. The ISA group's effort could serve as a model for a multidisciplinary approach to aquaculture.

A workshop was held during the International Symposium on the Brine Shrimp, Artemia (Corpus Christi, TX, 1979), in which characterization of the strains of Artemia for aquaculture was discussed (Simpson et al. 1980). As a result of the workshop, an Artemia reference sample was made available. This reference lot would serve as an internal control for aquaculturists (cf.

Sorgeloos, 1980). The reference cysts have been characterized and found to be suitable as food for marine fish and invertebrates. They are now available from the Artemia Reference Center, Ghent, Belgium. It is hoped that the same standardization can be achieved in the culture of other zooplankton which are fed to aquaculture species.

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## LITERATURE CITED


Andrews, J.W., L.V. Sick, and G.J. Baptist. 1972. The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. Aquaculture 1:341-347.

Beck, A.D., D.A. Bengtson, and W.H. Howell. 1980. International Study on Artemia. V. Nutritional value of five geographical strains of Artemia: effects on survival and growth of larval Atlantic silverside Menidia menidia. Pages 249-259 in G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), The Brine Shrimp Artemia, Vol. 3, Universa Press, Wetteren, Belgium.

Beck, A.D. and D.A. Bengtson. (In press). International Study of Artemia. XXII. Nutrition in aquatic toxicology: diet quality of geographical strains of the brine shrimp Artemia. Proceedings of the Fifth Annual Symposium on Aquatic Toxicology, October, 1980, American Society for Testing and Materials, Philadelphia, USA.

Benijts, F., E. VanVoorden, and P. Sorgeloos. 1975. Changes in the biochemical composition of the early larval stages of the brine shrimp, Artemia salina L. 10th European Symposium on Marine Biology, Ostend, Belgium. Vol. 1:1-9.

Bookhout, C.G. and J.D. Costlow, Jr. 1970. Nutritional effects of Artemia from different locations on larval development of crabs. Helgolander Wissenschafflich Meeresuntersuchungen 20:435-442.

Carlberg, J.M. and J.C. Van Olst. 1975. Brine shrimp (Artemia salina) consumption by the larval stages of the American

lobster (Homarus americanus) on relation to food density and water temperature. San Diego State University, Center for Marine Sciences. Contribution Number 14.

Claus, C., F. Benijts, and G. Vendeputte. 1979. The biochemical composition of the larvae of two strains of Artemia salina (L) reared on two different algal foods. Journal of Experimental Marine Biology and Ecology 36:171-183.

Colvin, P.M. 1976. Nutritional studies on penaeid prawns: protein requirements in compounded diets for juvenile Penaeus indicus (Milne Edwards). Aquaculture 7:315-326.

Cowey, D.B. 1979. Protein and amino acid requirements of finfish. In J.E. Halver and K. Tiews (eds.). Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology, Vol. 1, H. Heenemann and Co., Berlin.

Dabrowski, K. and J. Glogowski. 1977. Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. Hydrobiology 54:129-134.

Deshimaru, O. and K. Shigeno. 1972. Introduction to the artificial diet for prawn Penaeus japonicus. Aquaculture 1:115-133.

Deshimaru, O. and Y. Yone. 1978. Optimum level of dietary protein for prawn. Bulletin of the Japanese Society of Scientific Fisheries 44:1395-1397.

Detwyler, R. and E.D. Houde. 1970. Food selection by laboratory-reared larvae of the scaled sardine Harengula pensacolae (Pisces, Clupeidae) and the bay anchovy Anchoa mitchilli (Pisces, Engraulidae). Marine Biology 7:214-222.

Fujita, S. 1979. Culture of red sea bream, Pagrus major and its food. Pages 183-197 in E. Styczynska-Jurewitcz, T. Bachiel, E. Jaspers, and G. Persoone (eds.), Cultivation of Fish Fry and Its Live Food. European Mariculture Society Special Publication 4.

Gallagher, M. and W.D. Brown. 1975. Composition of San Francisco Bay brine shrimp (Artemia salina). Agriculture and Food Chemistry 23:630-632.

Grabner, M., W. Wieser, and R. Lackner. 1981. The suitability of frozen and freeze-dried zooplankton as food for fish larvae: a biochemical test program. Aquaculture 26:85-94.

Griffith, G.W., M.A. Kenslow, and L.A. Ross. 1973. A mass culture method for Tetraselmis sp., a promising food for larval crustaceans. Proceedings of World Mariculture Society 4:289-294.

Houde, E.D. 1973. Some recent advances and unsolved problems in the culture of marine fish larvae. Proceedings of the World Mariculture Society 3:83-112.

Howell, B.R. 1973. Marine fish culture in Britain - XIII. A marine rotifer, Brachionus plicatilis Muller, and the larvae of the mussel, Mytilus edulis L. as foods for larval flatfish. Journal du Conseil International Exploration de la Mer 35:1-6.

Hudinga, M. and M. Miyamura. 1962. Breeding of the "kuruma Prawn" (Penaeus japonicus Bate). Journal of the Oceanographic Society of Japan, 20th Anniversary Volume: 694-706.

Ito, T. 1960. On the culture of mixohalene rotifer Brachionus plicatilis O.F. Muller, in the seawater. Report Faculty of Fisheries, Prefectural University Mie 3:708-740.

Johns, D.M. and W. Walton. 1979. International Study of Artemia X. Effects of food source on survival, growth and reproduction in the mysid, Mysidopsis bahia. American Zoologist 19:906.

Johns, D.M., M.E. Peters, and A.D. Beck. 1980. International Study on Artemia. VI. Nutritional value of geographical and temporal strains of Artemia: effects on survival and growth of two species of brachyuran larvae. Pages 291-304 in G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), The Brine Shrimp Artemia, Vol. 3, Universa Press, Wetteren, Belgium.

Johns, D.M., W.J. Berry, and S. McLean. International Study of Artemia XXI. (in press). Why are some strains of Artemia better food sources than others? Further nutritional work with larvae of the mud crab, Rhithropanopeus harrisii. Proceedings of the World Mariculture Society.

Kanazawa, A., S.I. Teshima, and K. Ono. 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. comparative Biochemistry and Physiology 63B:295-298.

Ketola, H.G. 1976. Quantitative nutritional requirements of fishes for vitamins and minerals. Feedstuffs 48(7).

Kitajima, C., T. Arakawa, F. Oowa, S. Fujita, O. Imada, T.

Watanabe, and Y. Yone. 1980a. Dietary value for sea bream larvae of rotifer Brachionus plicatilis, cultured with baker's yeast, Saccharomyces cerevisiae, supplemented with cuttle fish liver oil. Bulletin of the Japanese Society of Scientific Fisheries 46:47-50.

Kinne, O. 1977. Marine Ecology Volume III. Cultivation. John wiley and Sons, London. 1293 pp.

Klein-MacPhee, G., W.H. Howell, and A.D. Beck. 1980. International Study on Artemia. VII. Nutritional value of five geographical strains of Artemia to winter flounder Pseudopleuronectes americanus larvae. Pages 305-312 in G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), The Brine Shrimp Artemia. Vol. 3. Universa Press, Wetteren, Belgium.

Lasker, R., H.M. Feder, G.H. Theilacker, and R.C. May. 1970. Feeding, growth, survival of Engraulis mordax larvae reared in the laboratory. Marine biology 5:345-353.

Lee, R.F. 1975. Lipids of arctic zooplankton. Comparative Biochemistry and Physiology 51B:263-266.

Lee, R.F. and J. Hirota. 1973. Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. Limnology and Oceanography 18:227-239.

McLean, S. 1980. The effect of cis-chlordane and dieldrin on the short food chain: Artemia to winter flounder. M.S. Thesis, University of Rhode Island, U.S.A.

Mock, C.R., D.B. Revera, and C.T. Fontaine. 1980. The larval

culture of Penaeus stylirostris using modifications of the Galveston laboratory technique. Proceedings of the World Mariculture Society 11:102-117.

Mootz, C.A. and C.E. Epifanio. 1974. An energy budget for Menippe mercenaria larvae fed Artemia nauplii. Biological Bulletin 146:44-55.

Moreno, V.J., J.E.A. DeMoreno, and R.R. Brenner. 1979a. Biosynthesis of unsaturated fatty acids in the diatom, Phaeodactylum tricornutum. Lipids 14:15-19.

Moreno, V.J., J.E.A. DeMoreno, and R.R. Brenner. 1979b. Fatty acid metabolism of the calanoid copepod Paracalanus parvus: 2 Palmitate, Stearate, Oleate and Acetate. Lipids 14:318-322.

Morris, R.J. and J.R. Sargent. 1973. Studies on the lipid metabolism of some oceanic crustaceans. Marine Biology 22:77-83.

Nash, C.E., C.M. Kuo, and S. McConnel. 1974. Operational procedures for rearing larvae of the grey mullet (Mugil cephalus L.). Aquaculture 3:15-24.

Olney, C.E., P.S. Schauer, S. McLean, You-Lu, and K.L. Simpson. 1980. International Study on Artemia. VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched Artemia and a laboratory-reared marine fish. Pages 343-352 in G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), The Brine Shrimp Artemia. Vol. 3, Universa Press, Wetteren, Belgium.

Patton, J.S. and A.A. Benson. 1975. A comparative study of wax ester digestion in fish. Comparative Biochemistry and

Physiology 52B:111-116.

Provenzano, A.J. and J.W. Goy. 1976. Evaluation of a sulphate lake strain of Artemia as a food for larvae of grass shrimp, Palaemonetes pugio. Aquaculture 9:343-350.

Rice, A.L. and D.I. Williamson. 1970. Methods for rearing larval decapod crustacea. Helgolander wissenschaftlich Meeresuntesuchen 20:417-434.

Samain, J.F., J. Moal, J.Y. Daniel, J.R. LeCoz, and M. Jezequel. 1980. The digestive enzymes amylase and trypsin during the development of Artemia: effect of food conditions. Pages 239-258 in G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), The Brine Shrimp Artemia. Vol. 2. Universa Press, Wetteren, Belgium.

Sargent, J.R. and R.F. Lee. 1975. Biosynthesis of lipids in zooplankton from Saanich Inlet, British Columbia, Canada. Marine biology 31:15-23.

Sargent, J.R., R. McIntosh, A. Bauermeister, and J.H.S. Blaxter. 1979. Assimilation of the wax esters of marine zooplankton by herring (Clupea harengus) and rainbow trout (Salmo gairdneri). Marine Biology 51:203-207.

Sastry, A.M. 1970. Culture of brachyuran crab larvae using a re-circulating seawater system in the laboratory. helgolander wissenschaftlich Meeresuntersuchen 20:406-416.

Schauer, P.S., L.M. Richardson, and K.L. Simpson. 1979. Nutritional status of marine larval and juvenile Menidia menidia reared on Artemia salina and various artificial diets. Pages 159-176 in E. Styczynska-Jurewicz, T. Bachiel,

E. Jaspers, and G. Persoone (eds.), Cultivation of Fish Fry and its Live Food. European Mariculture Society Special Publication 4.

Schauer, P.S., D.M. Johns, C.E. Olney, and K.L. Simpson. 1980. International Study on Artemia. IX. Lipid level, energy content and fatty acid composition of the cysts and newly-hatched nauplii from five geographical strains of Artemia. Pages 365-374 in G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), The Brine Shrimp Artemia. Vol. 3. Universa Press, Wetteren, Belgium.

Schauer, P.S. 1982. Bioaccumulation and bioconversion of dietary labeled fatty acids in the short food chain: rice bran to Artemia to rainbow trout and winter flounder. Ph.D. Thesis, University of Rhode Island.

Scott, A.P. and S.M. Baynes. 1978. Effect of algal diet and temperature on the biochemical composition of the rotifer, Brachionus plicatilis. Aquaculture 14:247-260.

Scott, A.P. and C. Middleton. 1979. Unicellular algae as a food for turbot (Scophthalmus maximus L.) larvae - the importance of dietary long-chain polyunsaturated fatty acids. Aquaculture 18:227-240.

Seidel, C.R., J. Kryznowek, and K.L. Simpson. 1980a. International Study on Artemia XI. Amino acid composition and electrophorectic protein patterns of Artemia from five geographical locations. Pages 375-382 in g. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), The Brine Shrimp Artemia. Vol. 3. Universa Press, Wetteren, Belgium.

Seidel, C.R., P.S. Schauer, T. Katayama, and K.L. Simpson. 1980b. Culture of Atlantic silversides fed on artificial diets and brine shrimp nauplii. Bulletin of the Japanese Society of Scientific Fisheries 46:237-245.

Simpson, K.L., A.D. Beck, and P. Sorgeloos. 1980. Report Workshop I: Characterization of Artemia strains for application in aquaculture. Pages 409-411 in G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.), The Brine Shrimp Artemia. Vol. 3. Universa Press, Wetteren, Belgium.

Simpson, K.L., T. Katayama, and C.O. Chichester. 1981. Carotenoids in fish feeds. In J.C. Bauernfeind (ed.), Carotenoids as Colorants and Vitamin A precursors. Academic Press, New York.

Soejima, T., T. Katayama, and K.L. Simpson. 1980. International Study on Artemia XII. The carotenoid composition of eight geographical strains of Artemia and the effect of diet on the carotenoid composition of Artemia. Pages 613-622 in G. Persoone, P. Sorgeloos, O. Roels and E. Jaspers (eds.), The Brine Shrimp Artemia. Vol. 2. Universa Press, Wetteren, Belgium.

Solangi, M.A. and J.T. Ogle. 1977. A selected bibliography on the mass propagation of rotifers with emphasis on the biology and culture of Brachionus plicatilis. Gulf Research Report 6(1):59-68.

Sorgeloos, P. 1980. Availability of reference Artemia cysts. Marine Ecology Progress Series 3:363-364.

Sorgeloos, P., R.P. McCourt, P. Lavens, L. Spectorova, and A.N.

Khalah. 1980a. Updated bibliography on the brine shrimp Artemia. European Mariculture Society Special Publication #5.

Sorgeloos, P., M. Baeza-mesa, E. Bossuyt, E. Bruggeman, J. Dobbeleir, D. Versichele, E. Lavina, and A. Bernardo. 1980b. Culture of Artemia on rice bran: the conversion of a waste-product into highly nutritive animal protein. Aquaculture 21:393-396.

Sulkin, S.D. and C.H. Epifanio. 1975. Comparison of rotifers and other diets for rearing early larvae of the blue crab, Callinectes sapidus Rathbun. Estuarine and coastal Marine Science 3:109-113.

Theilacker, G.H. and M.F. McMaster. 1971. Mass culture of the rotifer Brachionus plicatilis and its evaluation as a food for larval anchovies. Marine Biology 10:183-188.

VanHaecke, P. and P. Sorgeloos. 1980. International Study on Artemia. IV. The biometrics of Artemia strains from different geographical origin. Pages 393-405 in G. Persoone, P. Sorgeloos, O. Roels, and E. Jaspers (eds.), The Brine Shrimp Artemia. Vol. 3. Universa Press, Wetteren, Belgium.

Vankataramiah, A., G.J. Lakshmi, and G. Gunter. 1975. Effect of protein level and vegetable matter on growth and food conversion efficiency of brown shrimp. Aquaculture 6:115-125.

Watanabe, T., T. Arakawa, C. Kitajima, and S. Fujita. 1978a. Nutritional evaluation of proteins of living feeds used in seed production of fish. Bulletin of the Japanese Society of

Scientific Fisheries 44:985-988.

Watanabe, T., F. Oowa, C. Kitajima, and S. Fujita. 1978b. Nutritional quality of brine shrimp, Artemia salina as a living feed from the viewpoint of essential fatty acids for fish. Bulletin of the Japanese Society of Scientific Fisheries 44:1115-1121.

Watanabe, T., T. Arakawa, C. Kitajima, K. Fukusho, and S. Fujita. 1978c. Proximate and mineral composition of living feeds used in seed production of fish. Bulletin of the Japanese Society of Scientific Fisheries 44:979-984.

Watanabe, T., F. Oowa, C. Kitajima, S. Fujita, and Y. Yone. 1979. Relationship between the dietary value of rotifers, Brachionus plicatilis, and their content of ω3 highly unsaturated fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 45:883-889.

Watanabe, T., F. Oowa, C. Kitajima, and S. Fujita. 1980. Relationship between dietary value of brine shrimp, Artemia salina and their content of 3 highly unsaturated fatty acids. Bulletin of the Japanese Society of Scientific Fisheries 46:35-41.

Wickins, J.F. 1972. The food value of brine shrimp, Artemia salina L., to larvae of the prawn, Palaemon serratus Pennant. Journal of Experimental Marine Biology and Ecology 10:151-170.

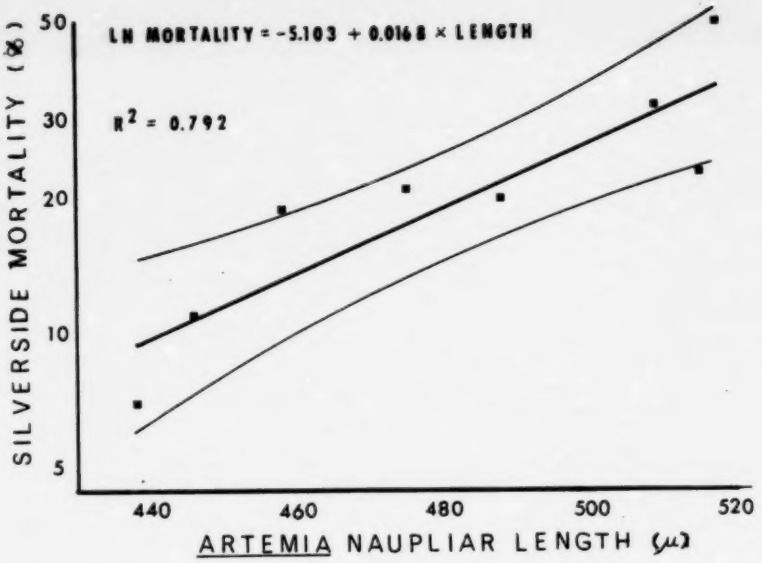


Figure 1. Natural logarithm of mortality of Atlantic silversides during the first five days of an experiment as a function of mean length of eight strains of Artemia nauplii fed to the fish. Bold, straight line is the regression line; thinner, curved lines represent 95% confidence limits.

Table I. Amino Acid Composition of Zooplankton as Compared with Penaeus japonicus (g/100 g crude protein)

| Amino acid | Artemia salina | | | Brachionus[d] plicatilis | Arcartia[d] clausi | Tigriopus[d] japonicus | Moina[d] spp. | Penaeus[e] japonicus |
|---|---|---|---|---|---|---|---|---|
| | #1[a] | #2[b] | #3[c] | | | | | |
| Isoleucine | 2.6 | 5.6 | 5.3 | 3.4 | 3.5 | 2.5 | 2.5 | 3.3 |
| Leucine | 6.1 | 8.9 | 8.0 | 6.1 | 5.5 | 5.0 | 6.0 | 5.5 |
| Methionine | 0.9 | 2.2 | 2.7 | 0.8 | 1.5 | 1.1 | 1.0 | 2.0 |
| Cystine | 0.4 | - | 2.2 | 0.6 | 0.8 | 0.7 | 0.6 | 0.8 |
| Phenylalanine | 3.2 | 5.1 | 4.7 | 3.9 | 3.7 | 3.5 | 3.6 | 3.3 |
| Tyrosine | 3.7 | 10.5 | 4.5 | 3.1 | 3.6 | 4.0 | 3.3 | 2.9 |
| Threonine | 1.7 | 5.2 | 4.6 | 3.2 | 4.2 | 3.8 | 3.8 | 3.0 |
| Tryptophan | 1.0 | - | 1.0 | 1.2 | 1.1 | 1.1 | 1.2 | - |
| Valine | 3.2 | 5.3 | 5.4 | 4.2 | 4.5 | 3.3 | 3.2 | 3.3 |
| Lysine | 6.1 | 11.7 | 7.6 | 6.1 | 5.4 | 5.7 | 5.8 | 5.8 |
| Arginine | 5.0 | 11.5 | 6.5 | 4.6 | 4.3 | 5.2 | 5.1 | 5.6 |
| Histidine | 1.3 | 4.9 | 1.8 | 1.5 | 1.9 | 1.6 | 1.6 | 1.6 |
| Alanine | 4.1 | 4.6 | 6.9 | 3.8 | 5.4 | 4.9 | 4.9 | 4.1 |
| Aspartic acid | 7.5 | 11.0 | 9.2 | 8.0 | 9.0 | 9.0 | 8.3 | 8.0 |
| Glutamic acid | 8.8 | 13.1 | 14.2 | 9.8 | 9.5 | 10.8 | 9.8 | 11.7 |
| Glycine | 3.4 | 6.0 | 5.3 | 3.1 | 4.6 | 4.5 | 3.7 | 3.4 |
| Proline | 4.7 | 5.7 | 5.2 | 6.7 | 4.6 | 4.8 | 4.2 | 2.6 |
| Serine | 4.6 | 4.5 | 4.8 | 4.0 | 3.3 | 4.3 | 4.0 | 3.0 |
| Total | 74.1 | | 99.9 | 74.1 | 76.4 | 75.8 | 72.6 | 69.9 |

[a]Newly-hatched; Watanabe et al. 1978a.

[b]Seidel et al. 1980b; Instar I stage, Macau, Brazil strain; expressed as % amino acids; tryptophan destroyed.

[c]Gallagher and Brown 1975.

[d]Watanabe et al. 1978a.

[e]Deshimaru and Shigeno 1972.

**Table II. Fatty Acid (as fatty acid methyl esters [FAME]) Composition of Newly Hatched *Artemia* (from Schauer et al. 1980)**

| FAME | Australia | Brazil | California | | | Italy | Utah |
|---|---|---|---|---|---|---|---|
| | | | SF (lot #313) | SF (lot #321) | SPB (lot #1628) | | |
| 14:0 | 1.34 | 1.57 | 0.99 | 1.57 | 0.43 | 1.53 | 0.93 |
| 14:1 | 2.23 | 0.81 | 1.27 | 0.74 | 2.26 | 3.30 | 1.45 |
| 15:0 | 0.34 | 0.67 | 0.16 | 0.58 | 0.25 | 0.11 | 0.11 |
| 15:1 | 0.15 | 0.24 | 0.20 | 0.13 | 0.46 | 0.54 | 0.37 |
| 16:0 | 13.45 | 15.42 | 10.33 | 12.13 | 7.79 | 15.23 | 11.78 |
| 16:1 | 9.97 | 10.79 | 13.27 | 19.52 | 5.24 | 10.38 | 5.64 |
| 16:2ω7 | ---[a] | --- | --- | --- | 1.51 | 2.94 | --- |
| 16:3ω4/17:1ω8 | 3.87 | 3.88 | 2.09 | 2.32 | 2.44 | 3.28 | 2.90 |
| 18:0 | 3.07 | 2.79 | 6.83 | 2.90 | 3.08 | 3.17 | 4.07 |
| 18:1ω9 | 28.23 | 35.86 | 26.97 | 31.20 | 29.15 | 29.05 | 28.58 |
| 18:2ω6 | 5.78 | 9.59 | 9.35 | 3.69 | 4.60 | 6.79 | 4.60 |
| 18:3ω3 | 14.77 | 4.87 | 17.33 | 5.16 | 33.59 | 6.35 | 31.46 |
| 18:4ω3 | 4.37 | 0.96 | 3.26 | 1.28 | 4.88 | 1.01 | 3.10 |
| 20:1ω9 | 0.37 | 0.52 | 0.41 | 0.35 | 0.35 | 0.42 | 0.37 |
| 20:2ω6/ω9 | 0.12 | 0.06 | 0.06 | --- | 0.24 | 0.20 | 0.09 |
| 20:3ω6 | 0.79 | 2.76 | 1.01 | 2.23 | 0.05 | 1.47 | 0.48 |
| 20:3ω3/20:4ω6 | --- | --- | 1.48 | 2.69 | 1.48 | --- | --- |
| 20:5ω3 | 10.50 | 8.98 | 4.06 | 12.44 | 1.68 | 13.63 | 3.55 |
| 22:6ω3 | 0.26 | 0.06 | --- | --- | --- | --- | --- |

[a]--- indicates the fatty acid was not found.

**Table III. Percent Distribution of 1-$^{14}$C 18:3ω3 in a Short Food Chain (from Schauer 1982)**

| Number double bonds | Labeled rice bran Diet #1[a] | *Artemia* fed labeled Diet #1[b] | Rainbow trout fed labeled *Artemia*[c] | Winter flounder fed labeled *Artemia*[c] |
|---|---|---|---|---|
| 0 + 1 | 0.21 | 8.80 | 8.29 | 8.07 |
| 2 | 0.04 | 3.26 | 19.2 | 24.2 |
| 3 | 98.5 | 68.2 | 23.1 | 20.5 |
| 4 | | 2.50 | 5.50 | 30.4 |
| 5 | 1.24 | 6.93 | 12.5 | 7.02 |
| 6 | | 10.30 | 31.4 | 9.80 |

[a]1-$^{14}$C 18:3ω3 was the only ω3 fatty acid in the diet.

[b]*Artemia* were fed the labeled diet for 7 days.

[c]Rainbow trout and winter flounder were fed the labeled *Artemia* for 3 days and starved for 3 days subsequent to the last feeding.

Table IV. Percentage Incorporation of [1-$^{14}$C] Linolenic Acid into the ω3 Pentaenes and Hexaenes of Lipids from Aquatic Animals Injected with [1-$^{14}$C] Linolenic Acid (from Kanazawa et al. 1979)

| Animal | Radioactive ω3 pentaenes and hexaenes formed from [1-$^{14}$C] linolenic acid | | |
|---|---|---|---|
| | Radioactivity (dpm x $10^4$) | % Incorporation[a] | Relative % incorporation to rainbow trout |
| Rainbow trout | 128.4 | 12.7 | 100 |
| Ayu | 6.5 | 4.5 | 36 |
| Eel | 11.3 | 2.5 | 20 |
| Red sea bream | 5.0 | 1.9 | 15 |
| Rockfish | 19.6 | 0.9 | 7 |
| Globefish | 25.8 | 1.6 | 13 |
| Prawn | 18.5 | 2.6 | 20 |

[a]% Incorporation of injected [1-$^{14}$C] linolenic acid to ω3 pentaenes and hexaenes comprising 20:5ω3, 22:5ω3, and 22:6ω3.

Table V. Fatty Acid (as fatty acid methyl esters [FAME]) Profiles of *Artemia* Nauplii and Adults Cultured in SEAFDEC Ponds (unpublished data)

| FAME | B-Nauplii[a] | B-Adults[c] | | SPB-Nauplii | SPB-Adults[c] | |
|---|---|---|---|---|---|---|
| | | 6/30/80[d] | 7/2/80[d] | (lot #1628) | 6/30/80[d] | 7/2/80[d] |
| 14:0 | 2.04 | 3.80 | 3.06 | 0.65 | 3.67 | 3.07 |
| 16:0 | 16.35 | 21.39 | 18.19 | 9.80 | 18.28 | 18.26 |
| 16:1 | 12.88 | 12.80 | 11.19 | 6.49 | 11.08 | 13.04 |
| 18:0 | 2.34 | 9.00 | 7.87 | 2.38 | 8.15 | |
| 18:1ω9 | 33.50 | 32.68 | 34.95 | 27.43 | 32.11 | 40.70 |
| 18:2ω6 | 9.17 | 9.07 | 8.95 | 5.30 | 10.74 | 9.24 |
| 18:3ω3 | 4.39 | 9.23 | 10.99 | 31.85 | 13.18 | 14.04 |
| 20:3ω6 | 2.30 | 0.69 | 1.15 | 0.04 | 0.98 | 0.08 |
| 20:5ω3 | 8.35 | 1.27 | 3.01 | 1.66 | 0.11 | 0.67 |

[a]Eggs from Macau, Brazil.

[b]Eggs from San Pablo Bay, California, USA.

[c]Adults cultured from Brazil (B) or San Pablo Bay (SPB) cysts.

[d]Collection date.

Table VI. The Amounts of β-Carotene, Echineone, Canthaxanthin and Zeaxanthin Present in *Artemia* after Feeding with *Spirulina* Pigments (from Soejima et al. 1980)

| Feeding program | Concentrations of carotenoids present (μg/g fresh wt) | | | | Unknown |
|---|---|---|---|---|---|
| | β-Carotene | Echinenone | Canthaxanthin | Zeaxanthin | |
| Group 1 | 0.11 | 5.87 | | | |
| Group 4 | 0.71 | 3.16 | 6.97 | 2.71 | 0.13 |
| Group 4' | 0.19 | 1.48 | 6.58 | 1.99 | |

Group 1: Rice bran only (12 days).

Group 4: Rice bran + pigments of *Spirulina* (6 days).

Group 4': Same as Group 4, then rice bran only (6 days).

**Table VII.** Metals (µg/g dry wt) in *Artemia* Cysts (C) and Nauplii (N) from Five Locations (from Olney et al. 1980)

| | Brazil | | Australia[a] | Italy | | Utah | | California | |
|---|---|---|---|---|---|---|---|---|---|
| | C | N | C | C | N | C | N | C | N |
| Fe | 804. | 62. | 870. | 1860. | 70. | 800. | 47. | 1380. | 46. |
| Zn | 59. | 89. | 144. | 125. | 104. | 81. | 102. | 78. | 98. |
| Pb | 8.9 | | 2.1 | 17. | 1.0 | 5.1 | 6.2 | 6.6 | 3.8 |
| Cu | 4.7 | 6.3 | 4.6 | 14. | 9.2 | 42. | 8.2 | 10.2 | 10.8 |
| Cd | 0.02 | 0.15 | 0.15 | 0.09 | 0.12 | 0.28 | 0.14 | 0.26 | 0.10 |
| Cr | | 1.4 | 1.8 | 3.7 | 0.66 | 2.0 | 1.1 | 4.4 | 0.48 |
| Ni | 0.23 | 0.29 | 0.50 | 4.0 | 0.09 | 2.9 | 0.70 | 6.6 | 0.12 |
| Co | 0.35 | 0.38 | 0.20 | 1.6 | 0.14 | 0.34 | | 1.9 | |
| Sc | 0.02 | | 0.01 | 0.41 | | 0.03 | | 0.31 | |
| Sb | 0.53 | | 0.53 | | | | | | |
| Se | 1.4 | | 1.2 | 1.2 | 1.0 | 1.5 | 2.0 | 2.3 | 0.83 |
| Rb | 6.5 | 12. | | | 7.5 | 23. | 23. | 9.1 | 6.5 |

[a]Nauplii from Australia not analyzed (insufficient sample).

**Table VIII.** Vitamin Composition of the Brine Shrimp *Artemia* as Compared to Minimum Dietary Requirement (MDR) of Salmonids

| Constituent | mg/g dry wt | |
|---|---|---|
| | Artemia[a] | Salmonid MDR[b] |
| Biotin | 0.001 | 0.00005 |
| Choline chloride | 6.10 | 0.05 |
| Folic acid | 0.001 | 0.001 |
| Niacin | 0.130 | 0.001 |
| Pantothenic acid | 0.068 | 0.01 |
| Pyridoxine HCl | 0.008 | 0.005 |
| Inositol | 1.20 | 0.25 |
| Riboflavine | 0.017 | 0.005 |
| Thiamine | 0.027[c] | 0.001 |
| Vitamin $B_{12}$ | 0.003 | - |
| Vitamin A | 14.6 IU/g[c] | 2.5 |
| Vitamin C | 0.049[c] | 0.050 |

[a]Gallagher and Brown (1975).

[b]Ketola (1976).

[c]M. Gallagher, personal communication (1982).

Table IX. Chlorinated Hydrocarbons (ng/g wet wt) in Artemia Nauplii (A) and the Mud Crab, Rhithropanopeus harrisii (R) (from Olney et al. 1980)

| Hydrocarbons | Brazil | | Australia | | Italy[a] | Utah | | California[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | SPB | | SFB (lot #313) | SFB (lot #321) |
| | A | R | A | R | A | A | R | A | R | A | A |
| HCB | 0.1 | 0.7 | 0.2 | ---[c] | 4.4 | 0.4 | 1.2 | 0.5 | 0.6 | 0.5 | 0.7 |
| α-BHC | 1.1 | 1.2 | 0.6 | 5.2 | 5.2 | 5.9 | 2.3 | 3.1 | 1.3 | 2.8 | 1.4 |
| γ-BHC | 0.8 | 0.2 | --- | 1.8 | 5.0 | 1.9 | 0.6 | 3.2 | 0.3 | 1.0 | 2.8 |
| c-chlordane | 0.1 | 0.1 | --- | 1.3 | 2.2 | 0.8 | 0.9 | 14.0 | 3.5 | 1.3 | 6.6 |
| t-nonachlor | <.1 | --- | --- | --- | --- | 1.2 | --- | 5.5 | --- | 1.4 | 8.3 |
| dieldrin | 0.2 | --- | 0.1 | --- | --- | 0.7 | 0.8 | 1.4 | 1.8 | 0.2 | 1.2 |
| op-DDE | 0.4 | --- | --- | --- | 3.9 | 0.7 | --- | 5.3 | 6.3 | 0.9 | 6.7 |
| pp-DDE | 1.2 | 2.4 | 1.3 | 6.1 | 100.0 | 2.2 | 4.0 | 18.0 | 19.0 | 2.0 | 10.0 |
| pp-DDD | 0.4 | --- | --- | --- | 74.0 | 3.0 | 2.1 | 13.0 | 6.5 | 3.8 | 22.0 |
| op-DDT | 0.4 | --- | 0.2 | 1.5 | 60.0 | 0.2 | --- | 1.0 | --- | --- | 2.0 |
| pp-DDT | 1.9 | 1.0 | 4.3 | 2.4 | 188.0 | 1.2 | 1.1 | 4.6 | 1.8 | 1.9 | 2.0 |
| Σ DDT | 4.3 | 3.4 | 5.8 | 10.0 | 422.0 | 7.3 | 7.2 | 42.0 | 33.6 | 8.7 | 43.0 |
| PCB (1016) | 5.3 | 21.0 | 2.0 | 29.0 | 5.3 | 3.2 | 30.0 | 14.0 | 16.0 | 6.4 | 18.0 |
| PCB (1254) | 1.6 | 14.0 | 0.9 | 20.0 | 19.0 | 6.5 | 20.0 | 29.0 | 33.0 | 3.5 | 23.0 |
| PCB (1260) | --- | 3.2 | --- | 2.0 | 7.5 | 4.9 | 6.1 | 22.0 | 25.0 | 3.5 | 2.8 |
| Σ PCB | 6.9 | 38.2 | 2.9 | 51.0 | 32.0 | 15.0 | 56.1 | 66.0 | 74.0 | 13.0 | 43.0 |
| % Survival | | 77% | | 61% | 75% (R) | | 0% | | 0% | | |

[a]No crabs available for Italy.

[b]San Pablo Bay, Calif. USA (SPB); San Francisco Bay, Calif. USA (SFB).

[c]--- indicates the hydrocarbon was not found.

[d]Johns et al. (1980); 1st crab stage.

Table X. Pesticide Concentrations (ng/g), Bioconcentration Factors (BCF) and Percent Uptake of the Pesticides in the Artemia (A) Treatment Groups (from McLean 1980)

| | Treatment group | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A-1,2 | A-3 | A-4 | A-5 | A-6 | A-7 | A-8 | A-9 | A-10 |
| Treatment[a] | Cont. | LC | HC | LD | HD | LC/LD | LC/HD | HC/LD | HC/HD |
| Pesticide conc. in water (ng/ml) | | | | | | | | | |
| cis-chlordane | 0 | 0.1 | 1.0 | 0 | 0 | 0.1 | 0.1 | 1.0 | 1.0 |
| dieldrin | 0 | 0 | 0 | 0.1 | 1.0 | 0.1 | 1.0 | 0.1 | 1.0 |
| ng Exposure | | | | | | | | | |
| cis-chlordane | 0 | 50 | 500 | 0 | 0 | 50 | 50 | 500 | 500 |
| dieldrin | 0 | 0 | 0 | 50 | 500 | 50 | 500 | 50 | 500 |
| Pesticide conc. in Artemia sp. (ng/g) | | | | | | | | | |
| cis-chlordane | 0 | 10.8[b] | 92.7 | 0 | 0 | 9.65 | 7.44 | 78.2 | 79.6 |
| | | 2.45[c] | 6.80 | | | 1.72 | 0.91 | 7.47 | 11.9 |
| dieldrin | 0 | 0 | 0 | 12.0 | 170.0 | 13.4 | 115.0 | 9.70 | 131.0 |
| | | | | 0.74 | 63.5 | 1.99 | 52.1 | 0.50 | 37.9 |
| BCF of Artemia sp. | | | | | | | | | |
| cis-chlordane | 0 | 108.0 | 92.7 | 0 | 0 | 96.5 | 74.4 | 78.2 | 79.6 |
| dieldrin | 0 | 0 | 0 | 120.0 | 171.0 | 134.0 | 115.0 | 96.9 | 131.0 |
| Percent uptake | | | | | | | | | |
| cis-chlordane | 0 | 21.7 | 18.5 | 0 | 0 | 19.3 | 14.9 | 15.6 | 15.9 |
| dieldrin | 0 | 0 | 0 | 24.1 | 34.2 | 26.8 | 23.1 | 19.4 | 26.2 |

[a]Low contamination (0.1 ppb) of cis-chlordane (LC); high contamination (1.0 ppb) of cis-chlordane (HC); low contamination (0.1 ppb) of dieldrin (LD); high contamination (1.0 ppb) of dieldrin (HD).

[b]Means of the two analytical replicates.

[c]Standard deviation of the two means.

Table XI. Temporal Study of Chlorinated Hydrocarbons in Utah *Artemia* Nauplii (from C. R. Seidel, personal communication)

| | 1965 | 1966 | 1970 | 1977 | 1978 |
|---|---|---|---|---|---|
| HCB | 0.32 | 0.32 | 0.62 | 0.73 | 0.49 |
| Σ PCB | 58.1 | 44.2 | 29.8 | 19.6 | 7.25 |
| Σ DDT | 96.8 | 51.5 | 27.3 | 22.9 | 11.6 |
| γ-BHC | 1.51 | 0.68 | 0.34 | 0.65 | 0.41 |
| Σ Chlordanes | 4.1 | 4.3 | 8.2 | 4.2 | 4.5 |

Table XII. Summary of the Biological Response of Several Fish and Shellfish to Five Geographical Strains of *Artemia*. (+ = good survival, - = poor survival, ± = good survival but poor growth)

| | Brazil | Australia | Utah | SPB | Italy |
|---|---|---|---|---|---|
| *Cancer irroratus*[a] | + | + | - | - | + |
| *Rhithropanopeus harrisii*[a] | + | + | - | - | + |
| *Mysidopsis bahia*[b] | + | + | + | ± | + |
| *Menidia menidia*[c] | + | + | + | ± | + |
| *Pseudopleuronectes americanus*[d] | + | + | - | - | + |

[a]From Johns et al. (1980). [b]From Johns and Walton (1979).

[c]From Beck et al. (1980). [d]Klein-MacPhee et al. (1980).